REMARKS

Claims 1-12, 14 and 16-18 are pending. Claims 1-11 are withdrawn from consideration.

Claims 12 and 14-17 stand rejected under 35 U.S.C. 112, as being indefinite.

Applicants urge that it is not mandatory for the claimed process that only subterminally hydroxylated products are obtained. As illustrated by the experimental results, summarized in Table 4 of the present specification, the present invention allows (if compared to the wild-type enzyme) a shift of the hydroxylation position as illustrated for the C_{12}-carboxylic acid lauric acid. Moreover, the present invention allows for the first time the subterminal hydroxylation of short-chain carboxylic acids with less than 12 carbon atoms as illustrated in Table 4 for the C_{10}-carboxylic acid capric acid. In the context of the present invention it is, therefore, completely immaterial whether or not as a by-product terminally hydroxylated products might be obtained as well. It should be sufficient to describe a claimed process by stating the preparation of the desired products (rather than unwanted by-products) if the invention is based on the preparation of said desired products.

Moreover, a skilled person may rely on routine methods in order to separate the obtained reaction mixtures. A separation for analytical purposes is illustrated by "method 6" (see pages 17 and 18 of the specification). Moreover, a skilled reader is perfectly aware of the fact that chromatographic methods as for example reversed-phase chromatography may be applied to isolate the desired hydoxylation products. For example, the disclosure of Graham-Lorence, section "Material and Methods" as well as to the disclosure of Olivier et al. which corresponds to document (13) mentioned on page 19, line 18 of the application text. Olivier et al. suggest for product identification reversed-phase HPLC (see section "Experimental Procedures", subsection "Product Identification").

Applicants have amended the claims to overcome the other points of rejection under 35 U.S.C. 112, second paragraph.

Claims 12 and 16-17 stand rejected under 35 U.S.C. 112, first paragraph. Applicants respectfully traverse this rejection. Applicants have further specified in amended claim 12 the preferred amino acid sequence positions to be mutated, in response to the Examiner's argument that the claimed method encompasses the use of any modified cytochrome P450 monooxygenase. Moreover, claim 12 is restricted to the subterminal hydroxylation of carboxylic acids of medium chain length (C_8-C_{12}-carboxylic acids) and specific derivatives thereof.

Moreover, we are of the opinion that the requirements to be considered in determining whether undue experimentation is required are met. In particular, the disclosure of the present invention is not limited to a method for hydroxylating para-nitrophenoxy derivatised carboxylic acids as speculated by the Examiner on page 8 of the Office Action.

As already mentioned, Table 4 summarizes experimental evidence obtained for a representative number of different single or multiple mutants of the present invention as well as for the wild-type enzyme when applied in the hydroxylation of non-derivatised carboxylic acids of different chain lenghts (capric acid and lauric acid). The mutants applied according to Example 4, Table 4 meet the structural requirements as defined by new claim 12. Therefore, sufficient experimental evidence for reproducing the claimed invention without undue experimentation is provided by the present specification.

Moreover, there is a considerable amount of guidance disclosed in the experimental part of the present specification. In addition to the general method section (see pages 13 to 19 of the specification) Examples 1, 2 and 3 disclose in detail how the different embodiments of the present invention are obtainable by a skilled reader. Example 1 explains the selection of a

suitable starting mutant with improved specific activity for carboxylic acids with the desired short chain length of 8 to 12 carbon atoms. Example 2 explains the selection of individual positions of mutation with the capability of forming new carboxylate binding sites for short-chain carboxylic acids. Example 3 discloses the stepwise production of multiple mutants. Example 4, as explained above, refers to the testing of the obtained mutants with carboxylic acids of different chain length and compares said mutants to the wild-type enzyme.

In view of said plethora of experimental details originally disclosed in the specification, applicants urge that the invention as claimed has been adequately enabled.

Claims 12, and 14-17 stand rejected under 35 U.S.C. 102(b) as being anticipated by Graham-Lorence et al. Applicants respectfully traverse this rejection.

Graham-Lorence et al. do not disclose the subterminal hydroxylation of C_8-C_{12}-carboxylic acid. The substrate as applied by Graham-Lorence is a carboxylic acid with a chain length of 20 carbon atoms (arachidonic acid). Moreover, a person of ordinary skill will recognize that arachidonic acid is not a derivative of a C_8-C_{12}-carboxylic acid in the sense of new claim 12.

Claims 12, and 16-17 stand rejected under 35 U.S.C. 102(b) as being anticipated by Schwaneberg et al. Applicants respectfully traverse this rejection.

The disclosure of Schwaneberg is limited to the single mutant F87A, which is not encompassed by the definition of amended claim 12. Moreover, said single mutation in position 87 (F to A) results in a shift of the preferred hydroxylation position from the subterminal to the terminal ω-position as stated by Schwaneberg on page 365, left column, 3rd paragraph, lines 8 to 10. Contrary to this, the mutants of the present invention cause a more pronounced hydroxylation in the ω-2 or ω-3 position if compared to the wild-type enzyme (see data obtained

for lauric acid summarized in table 4 on page 25 of the specification).

In this respect applicants also refer to the disclosure of Olivier et al. (cited by the Examiner in the first Office Action). As confirmed by Olivier et al. (see Abstract on page 1567, last two lines), said F87A mutant catalyzes hydroxylation almost exclusively at the ω-position in marked contrast to the wild-type enzyme, with which no hydroxylation at this position was observed.

Therefore, applicants urge that the claimed subject matter is new over Graham Lorence et al. or Schwaneberg et al.